Possibility, duration, and molecular predictors of sanitizer tolerance in *Listeria monocytogenes*

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Project funding dates

January 1, 2020 – December 31, 2021

Acknowledgements

We thank Dr. Yi Chen, Dr. Sophia Kathariou and Dr. Hailey Oliver for providing bacterial strains.

Summary

Adaptation to sub-lethal concentrations of benzalkonium chloride (BC) induces additional BC resistance in both inherently resistant and sensitive *Listeria monocytogenes* (Lm) strains. The degree, duration, and molecular mechanism of acquired resistance differ between strain H7550 and its plasmid-cured derivative H7550-Cd^s. Through exposure to BC at 50% of inherent minimum inhibitory concentration (MIC), both strains showed acquired resistance after 9 h and became fully adapted to tolerate higher levels of BC after 24 h for H7550-Cd^s (5-fold MIC increase) and 72 h for H7550 (1.5-fold MIC increase). Acquired resistance persisted in adapted H7550-Cd^s and its progenies but immediately subsided in adapted H7550 after removal of BC exposure. Gradual adaptation to increasing BC did not appear to induce higher acquired resistance than constant exposure at 50% inherent MIC for both strains.

Methods

Lm strains were adapted on Mueller Hinton agar with 2% defibrinated sheep blood containing variable concentrations of benzalkonium chloride (BC). Lm strains with (H7550) and without (H7550-Cd^s) *bcrABC* cassette were selected to measure their acquired resistance after either short-term (up to 24 h with 3-h intervals) or long-term (up to 4 d) adaptation to sub-lethal levels of BC (50% of inherent MIC). Adapted Lm strains were transferred to 0.1% BPW without BC for up to 4 d to measure the duration of acquired resistance. In addition to exposure to constant levels of BC, the Lm strains also were adapted to increasing concentrations of BC and measured for acquired resistance (**Fig. 1**).

Objectives

1. Survey of residual sanitizer concentrations in selected locations in two different produce processing facilities between sanitation shifts.
2. Measurement of intrinsic tolerance to sodium hypochlorite and benzalkonium chloride in 200–300 strategically selected *Listeria monocytogenes* (Lm) strains.
3. **Evaluation of how different levels of sanitizers and lengths of sanitizer exposure affect the degree and duration of acquired sanitizer tolerance in selected Lm strains.** – *focus for work from 6/2020 to 6/2021*
4. Characterization of transcriptomic shifts that accompany the waning of acquired sanitizer tolerance.
5. Whole genome sequencing (WGS) analyses of Lm to (1) develop machine-learning classifiers for intrinsic sanitizer tolerance prediction, and (2) search for evolutionary evidence for intrinsic tolerance development.

Results to Date

When exposed to a constant level of BC (50% of inherent MIC), acquired resistance emerged after 9 h in both H7550-Cd^s and H7550. In H7550-Cd^s, acquired resistance fully developed after 24 h, with the MIC increasing from 5 to 35 μg/ml (**Fig. 2A**). In H7550, acquired resistance fully developed after 72 h, with its MIC increasing from 30 to 50 μg/ml (**Fig. 2B**).

In H7550-Cd^s, acquired resistance persisted after removal of BC exposure (Fig. 2A) and culturing of the adapted strain (data not shown). In H7550, acquired resistance subsided substantially immediately after removal of BC exposure (**Fig. 2B**).

When exposed to increasing levels of BC, H7550-Cd^s acquired tolerance up to ~35 μg/ml (**Fig. 3A**), while H7550 showed varying levels of acquired resistance, which were not higher than induced by adapting to BC at 50% MIC (**Fig. 3B**).

Benefits to the Industry

The results of this project may (i) help determine the frequency of BC sanitation to minimize the development of acquired resistance in both inherently sensitive and resistant strains, and (ii) provide further evidence that significantly higher BC resistance may be permanently acquired by originally sensitive strains through a short (e.g., 24 h), sub-lethal exposure to BC, which may involve genetic mutation(s).

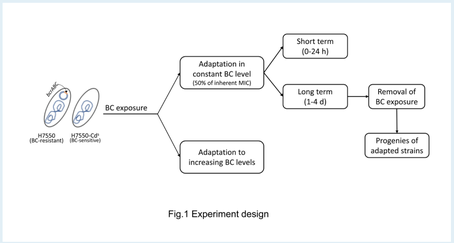

Fig.1 Experiment design

Figure 1

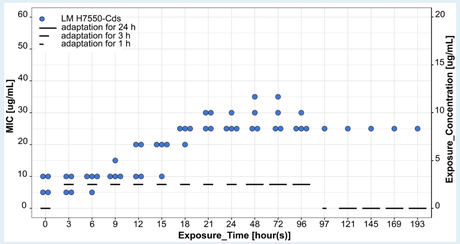

Figure 2A

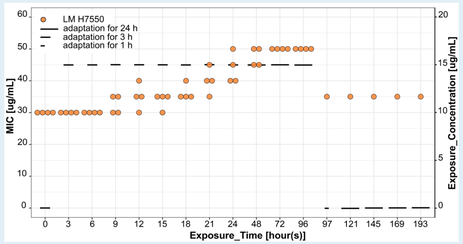

Figure 2B

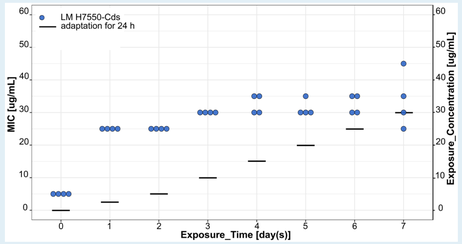

Figure 3A

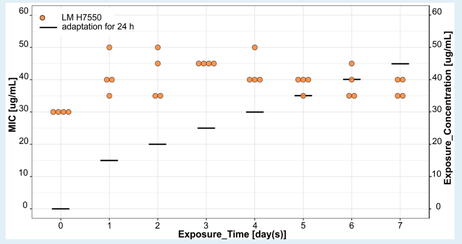

Figure 3B